

IN THE SPECIFICATION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application is a divisional of United States Application No. 09/614,748, filed on July 12, 2000, now U.S. Patent 6,660,474, which is a continuation of International Application No. PCT/US99/00663, filed on January 12, 1999, which claims the benefit of United States Provisional Application No. 60/071,199, filed on January 12, 1998, and United States Provisional Application No. 60/098,279, filed on August 28, 1998, the disclosures of which are incorporated by reference herein.

[0005] It has been estimated that genetic factors account for 30-40% of blood pressure variability in humans (Ward, In *Hypertension: Pathophysiology, Diagnosis and Management*, Laragh JH. and Brenner BM eds., (Raven Press, Ltd., New York, NY), 81-100 (1990).) However, other estimates have suggested that genetic heritability of hypertension may be as high as 80% with 40% accounted for by one major gene (Cavalli, et al., ~~In~~in *The Genetics of Human Population*, (WH Freeman Co., South San Francisco, CA) 534-536 (1971)). The single major gene could effect blood pressure to such a significant extent that it would dominate many other genes that play a minor role in blood pressure control.

~~BRIEF DESCRIPTION OF THE DRAWINGS~~

[0017] Without being bound by any particular theory of operation, Applicants believe that a renal defect is responsible for a certain portion of hypertension in human subjects, and that the *GRK4* mutation either causes among other things, a direct or indirect ligand independent serine-hyperphosphorylation of the D1 receptor, resulting in its uncoupling from the G protein/effector complex. The result is that the natriuretic effect of dopamine is compromised and the

kidney is unable to properly balance sodium and water, leading to sodium retention and elevated blood pressure. More specifically, renal proximal tubules obtained from human hypertensive subjects, but not from normotensive subjects, demonstrate a defective coupling of the dopamine D1 receptor with adenylyl cyclase. The defective coupling is associated with a ligand-independent phosphorylation of the D1 receptor. Applicants have discovered at least six mutations in G protein related kinase type 4 (GRK4), that regulate ligand-independent phosphorylation of the D1 receptor in hypertensive patients.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] GRK4 is originally reported in Ambrose, et al., Hum. Mol. Genet. 1:697-703 (1993), and then more extensively characterized in Premont et al., J. Biol. Chem. 271(11):6403-6410 (1996). Premont reports that GRK4 is highly abundant in testis only, GRK4 mRNA being present to a small extent in brain and skeletal muscle. The GRK4 gene, exclusive of promoter regions, spans approximately 75 kilobases (kDa), and is composed of 16 exons. The longest form of GRK4, with intact amino- and carboxyl-terminal alternative exon sequences, has been designated GRK4alpha. The deduced protein sequence contains 578 amino acids, with a predicted molecular mass of 66.5 kDa. The next shorter form, GRK4beta, lacks only the amino-terminal alternative exon, which is composed of codons, and thus contains 546 amino acids having a molecular mass of 62.kDa. GRK4 gamma is the isoform lacking only the carboxyl-terminal alternative exon, which is 46 codons. Thus, this isoform contains 532 amino acids, and has a predicted molecular mass of 61.2 kDa. GRK4gamma was formally called GRK4A. See Salles et al., Biochem. Biophys. Res. Commun. 199:848-854 (1994). GRK4delta contains 500 amino acids with a predicted molecular mass of 57.6kDa, and is the shortest isoform. It lacks both alternative exons. GRK4delta was originally

designated IT11 and GRK4B. See Sallese *et al.*, *supra*, and Ambrose, *et al.*, *supra*. More recently, two additional isoforms have been discovered, namely: GRK4epsilon which lacks exons 13 and 15, contains ~~466~~486 amino acids with a predicted molecular mass of 53.6 kDa, and GRK4zeta which lacks exons 2, 13 and 15, contains ~~434~~454 amino acids with a predicted molecular mass of 49.9 kDa.

[0025] Five single nucleotide polymorphisms of *GRK4* are also known, namely: R65L (CGT to CTT); A142V (GCC to GTC); V247I (GTA to ATA); A486V (GCG to GTG) and D562G (GAC to GGC). See Premont, *et al.*, *supra*. Applicants have discovered that the R61L, the A142V and the A486V polymorphisms are associated with essential hypertension. Applicants have also discovered three additional polymorphisms prevalent in hypertensive individuals, namely: the double mutants R65L, A142V and R65L, A486V; and the triple mutant R65L, A142V, A486V. Table 1 shows the amino acid and corresponding nucleotide sequences of the six GRK4 isoforms. Amino acids and corresponding nucleotides that are changed in the polymorphs associated with essential hypertension are shown in bold. The sequences of the 5' untranslated regions of the epsilon and Zeta isoforms are not shown.

Table 1

MELLENIVANS	LLLKARQGGY	GKKSGRSKKW	KEILTLPPVS	QCSELRHSIE	50	GRK4 α
MELLENIVANS	LLLKARQ----	-----	-----	-----		GRK4 β
		-----	-----	-----E		
MELLENIVANS	LLLKARQGGY	GKKSGRSKKW	KEILTLPPVS	QCSELRHSIE		GRK4 γ
MELLENIVANS	LLLKARQ----	-----	-----	-----		GRK4 δ
		-----	-----	-----E		
MELLENIVANS	LLLKARQGGY	GKKSGRSKKW	KEILTLPPVS	QCSELRHSIE		GRK4 ϵ
MELLENIVANS	LLLKARQ----	-----	-----	-----		GRK4 ζ
		-----	-----	-----E		
KDYSSLCDKQ	PIGRRLFRQF	CDTKPTLKRH	IEFLDAVAEY	EVADEDRSD	100	GRK4 α
KDYSSLCDKQ	PIGRRLFRQF	CDTKPTLKRH	IEFLDAVAEY	EVADEDRSD		GRK4 β
KDYSSLCDKQ	PIGRRLFRQF	CDTKPTLKRH	IEFLDAVAEY	EVADEDRSD		GRK4 γ
KDYSSLCDKQ	PIGRRLFRQF	CDTKPTLKRH	IEFLDAVAEY	EVADEDRSD		GRK4 δ
KDYSSLCDKQ	PIGRRLFRQF	CDTKPTLKRH	IEFLDAVAEY	EVADEDRSD		GRK4 ϵ
KDYSSLCDKQ	PIGRRLFRQF	CDTKPTLKRH	IEFLDAVAEY	EVADEDRSD		GRK4 ζ
CGLSILDRFF	NDKLAAPLPE	IPPDVVTECR	LGLKEENPSK	KAFFECTRVA	150	GRK4 α
CGLSILDRFF	NDKLAAPLPE	IPPDVVTECR	LGLKEENPSK	KAFFECTRVA		GRK4 β
CGLSILDRFF	NDKLAAPLPE	IPPDVVTECR	LGLKEENPSK	KAFFECTRVA		GRK4 γ
CGLSILDRFF	NDKLAAPLPE	IPPDVVTECR	LGLKEENPSK	KAFFECTRVA		GRK4 δ
CGLSILDRFF	NDKLAAPLPE	IPPDVVTECR	LGLKEENPSK	KAFFECTRVA		GRK4 ϵ
CGLSILDRFF	NDKLAAPLPE	IPPDVVTECR	LGLKEENPSK	KAFFECTRVA		GRK4 ζ
HNYLRGEPFE	EYQESSYFSQ	FLQWKWLERQ	PVTKNTRHY	RVLGKGGFGE	200	GRK4 α
HNYLRGEPFE	EYQESSYFSQ	FLQWKWLERQ	PVTKNTRHY	RVLGKGGFGE		GRK4 β
HNYLRGEPFE	EYQESSYFSQ	FLQWKWLERQ	PVTKNTRHY	RVLGKGGFGE		GRK4 γ
HNYLRGEPFE	EYQESSYFSQ	FLQWKWLERQ	PVTKNTRHY	RVLGKGGFGE		GRK4 δ
HNYLRGEPFE	EYQESSYFSQ	FLQWKWLERQ	PVTKNTRHY	RVLGKGGFGE		GRK4 ϵ
HNYLRGEPFE	EYQESSYFSQ	FLQWKWLERQ	PVTKNTRHY	RVLGKGGFGE		GRK4 ζ
VCACQVRATG	KMYACKKLQ	KRIKKRKGEA	MALNEKRILE	KVQSRFVVSL	250	GRK4 α
VCACQVRATG	KMYACKKLQ	KRIKKRKGEA	MALNEKRILE	KVQSRFVVSL		GRK4 β
VCACQVRATG	KMYACKKLQ	KRIKKRKGEA	MALNEKRILE	KVQSRFVVSL		GRK4 γ
VCACQVRATG	KMYACKKLQ	KRIKKRKGEA	MALNEKRILE	KVQSRFVVSL		GRK4 δ
VCACQVRATG	KMYACKKLQK	KRIKKRKGEA	MALNEKRILE	KVQSRFVVSL		GRK4 ϵ
VCACQVRATG	KMYACKKLQ	KRIKKRKGEA	MALNEKRILE	KVQSRFVVSL		GRK4 ζ

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AYAYETKDAL	CLVLTIMNGG	DLKFHIYNLG	NPGFDEQRAV	FYAAELCCGL	300	GRK4 α
AYAYETKDAL	CLVLTIMNGG	DLKFHIYNLG	NPGFDEQRAV	FYAAELCCGL		GRK4 β
AYAYETKDAL	CLVLTIMNGG	DLKFHIYNLG	NPGFDEQRAV	FYAAELCCGL		GRK4 γ
AYAYETKDAL	CLVLTIMNGG	DLKFHIYNLG	NPGFDEQRAV	FYAAELCCGL		GRK4 δ
AYAYETKDAL	CLVLTIMNGG	DLKFHIYNLG	NPGFDEQRAV	FYAAELCCGL		GRK4 ϵ
AYAYETKDAL	CLVLTIMNGG	DLKFHIYNLG	NPGFDEQRAV	FYAAELCCGL		GRK4 ζ
EDLQERIVY	RDLKPENILL	DDRGHIRISD	LGLATEIPEG	QVRGRVGTV	350	GRK4 α
EDLQERIVY	RDLKPENILL	DDRGHIRISD	LGLATEIPEG	QVRGRVGTV		GRK4 β
EDLQERIVY	RDLKPENILL	DDRGHIRISD	LGLATEIPEG	QVRGRVGTV		GRK4 γ
EDLQERIVY	RDLKPENILL	DDRGHIRISD	LGLATEIPEG	QVRGRVGTV		GRK4 δ
EDLQERIVY	RDLKPENILL	DDRGHIRISD	LGLATEIPEG	QVRGRVGTV		GRK4 ϵ
EDLQERIVY	RDLKPENILL	DDRGHIRISD	LGLATEIPEG	QVRGRVGTV		GRK4 ζ
GYMAPEVNN	EKYTFSPDWW	GLGCLİYEMI	QGHSPFKKYK	EKVKWEEVDQ	400	GRK4 α
GYMAPEVNN	EKYTFSPDWW	GLGCLİYEMI	QGHSPFKKYK	EKVKWEEVDQ		GRK4 β
GYMAPEVNN	EKYTFSPDWW	GLGCLİYEMI	QGHSPFKKYK	EKVKWEEVDQ		GRK4 γ
GYMAPEVNN	EKYTFSPDWW	GLGCLİYEMI	QGHSPFKKYK	EKVKWEEVDQ		GRK4 δ
GYMAPEVNN	EKYTFSPDWW	GLGCLİYEMI	QGHSPFKKYK	EKVKWEEVDQ		GRK4 ϵ
GYMAPEVNN	EKYTFSPDWW	GLGCLİYEMI	QGHSPFKKYK	EKVKWEEVDQ		GRK4 ζ
RIKNDTEEYS	EKFSEDAKSI	CRMLLTKNPS	KRLGCRGEGA	AGVKQHPVFK	450	GRK4 α
RIKNDTEEYS	EKFSEDAKSI	CRMLLTKNPS	KRLGCRGEGA	AGVKQHPVFK		GRK4 β
RIKNDTEEYS	EKFSEDAKSI	CRMLLTKNPS	KRLGCRGEGA	AGVKQHPVFK		GRK4 γ
RIKNDTEEYS	EKFSEDAKSI	CRMLLTKNPS	KRLGCRGEGA	AGVKQHPVFK		GRK4 δ
RIKNDTEEYS	EKFSEDAKSI	CRM-----	-----	-----		GRK4 ϵ
RIKNDTEEYS	EKFSEDAKSI	CRM-----	-----	-----		GRK4 ζ
DINFRRLEAN	MLEPPFCPDP	HAVYCKDVLD	IEQFS AV KGI	YLDTADED F Y	500	GRK4 α
DINFRRLEAN	MLEPPFCPDP	HAVYCKDVLD	IEQFS AV KGI	YLDTADED F Y		GRK4 β
DINFRRLEAN	MLEPPFCPDP	HAVYCKDVLD	IEQFS AV KGI	YLDTADED F Y		GRK4 γ
DINFRRLEAN	MLEPPFCPDP	HAVYCKDVLD	IEQFS AV KGI	YLDTADED F Y		GRK4 δ
-----	-----P	HAVYCKDVLD	IEQFS AV KGI	YLDTADED F Y		GRK4 ϵ
-----	-----P	HAVYCKDVLD	IEQFS AV KGI	YLDTADED F Y		GRK4 ζ
ARFATGCVSI	PWQNEMIESG	CFKDINKSES	EEALPLDL DK	NIHTPVSRPN	550	GRK4 α
ARFATGCVSI	PWQNEMIESG	CFKDINKSES	EEALPLDL DK	NIHTPVSRPN		GRK4 β
ARFATGCVSI	PWQNE-----	-----	-----	-----		GRK4 γ
ARFATGCVSI	PWQNE-----	-----	-----	-----		GRK4 δ

ARFATGCVSI	PWQNE-----	-----	-----	-----	GRK4ε
ARFATGCVSI	PWQNE-----	-----	-----	-----	GRK4ζ
RGFFYRLFRR	GGCLTMVPSE	KEVEPKQC	578	GRK4α	(SEQ ID NO:1)
RGFFYRLFRR	GGCLTMVPSE	KEVEPKQC	546	GRK4β	(SEQ ID NO:2)
-----	-GCLTMVPSE	KEVEPKQC	532	GRK4γ	(SEQ ID NO:3)
-----	-GCLTMVPSE	KEVEPKQC	500	GRK4δ	(SEQ ID NO:4)
-----	-GCLTMVPSE	KEVEPKQC	<u>466486</u>	GRK4ε	(SEQ ID NO:5)
-----	-GCLTMVPSE	KEVEPKQC	<u>434454</u>	GRK4ζ	(SEQ ID NO:6)

Note: The bolded letters indicate the change in amino acid associated with hypertension R to L (arginine to leucine), A to V (alanine to valine), and A to V (alanine to valine).